NASA TTF-10, 258

SEPARATION OF SIMPLE SUGARS ON CELLULOSE LAYERS

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Translation of "Trennung einfacher Zucker auf Cellulose-Schichten". Journal of Chromatography, Vol. 9, pp. 374-376, 1962.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON D.C. AUGUST 1966

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ABSTRACT

Polysacchride hydrolysates were investigated with respect to their content in sugar and uronic acids. The following motion substances were used: acetic acid, ethyl ester-pyridine-water, phenol saturated with water and isopropanol-pyridine-glacial acetic acid-water. Very small amounts of substance can be identified with the method.

Thin-layer chromatography has been successfully applied to various /374* disciplines on the chemistry of natural materials (Ref. 1). A recent publication dealt with trace analysis of sugars using layers of silicagel (Ref. 2). The present publication discusses the chromatographic separation of sugars on a thin layer of cellulose powder. The procedure was worked out for the identification of the hydrolysis products of the polysaccharides of plants. The goals and results of these experiments will be discussed elsewhere (Ref. 3).

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* Note: Numbers in the margin indicate pagination in the original foreign text.

Description of the Method

In general, we proceeded according to a perspectus of the firm Macherey, Nagel, and Company, Duren. This firm also supplied the cellulose powder (cellulose powder MN 300). 15 g powder was homogenized with 90 ml aqua dest. for about 30 seconds in the Starmix. It was not necessary to add gypsum or other binding agents. The cellulose paste was applied with a layer thickness of 0.25 mm. For this purpose, we used plates and a spreading apparatus, which are standard tools for thin layer chromatography of the firm Desaga, Heidelberg. The wet layer was dried for 10 minutes at about 100° in the dry chamber.

For purposes of chromatography, the substances are applied at distances of 2 cm along a starting line located 3 cm from the lower edge of the plates.

The following mixtures were used as the means of motion:

I. Acetic acid ethyl ester-pyridine-water (2:1:2), both phases (Ref. 4).

II. Phenol saturated with water -- 1% ammonia (Ref. 5).

III. Isopropanol-pyridine-glacial acetic acid-water (80:80:10:40) (Ref. 6).

The chromatography is carried out in glass vessels furnished by the firm Desaga for "chamber oversaturation" (Ref. 7). The plate is lifted from the vessel when the front of the motion substance reaches the upper edge, and it is then dried. When the mixture I was used, the plate was usually introduced into the vessel again for a second course in the same direction, and using the same motion substance. The method requires about 4 hours for two-fold chromatography.

The sugars can be colored with anisidine phthalate (0.1 M solution

p-anisidine and phthalic acid in 96% ethanol) (Ref. 8). Hexoses are colored green, pentoses red-violet, methyl pentoses yellow-green, and uronic acids brown.

Results

For the most part, the sugars and uronic acids shown in Table 1 occured in the polysaccharide hydrolysates investigated.

As can be seen from the motion values, referring to distance travelled by the glucose (R_g values), the monosaccharide galactose, glucose, mannose, xylose, ribose and rhamnose can be separated well when the motion substance I is used (See Figure 1). Only the spots of mannose and rhabinose are situated very close together. These sugars can be completely separated by means of rechromatography using the motion substance II.

Sulser suggested the motion substance III (Ref. 6) for paper chromatography of uronic acids.We applied it to a thin layer and obtained a satisfactory separation of glucoroic, mannuronic, and galacturonic acids.

TABLE 1 /375

R_G-VALUES OF THE SUGARS AFTER TOW FOLD CHROMATOGRAPHY ON A LAYER OF CELLULOSE POWDER (SYSTEM 1)

D(+)-Glucose	1.00
D(+)-Galactose	0.90
D(+)-Mannose	1.09
L(+)-Arabinose	1.11
D(+)-Xylose	1.25
D(—)-Ribose	1.42
L(+)-Rhamnose	1.52
D(+)-Glucuronsäure (1)	—
D(+)-Galacturonsäure (2)	—
D(+)-Mannuronsäure (3)	—

1- Glucoroic, 2- Galacturonic, 3- Mannuronic acids

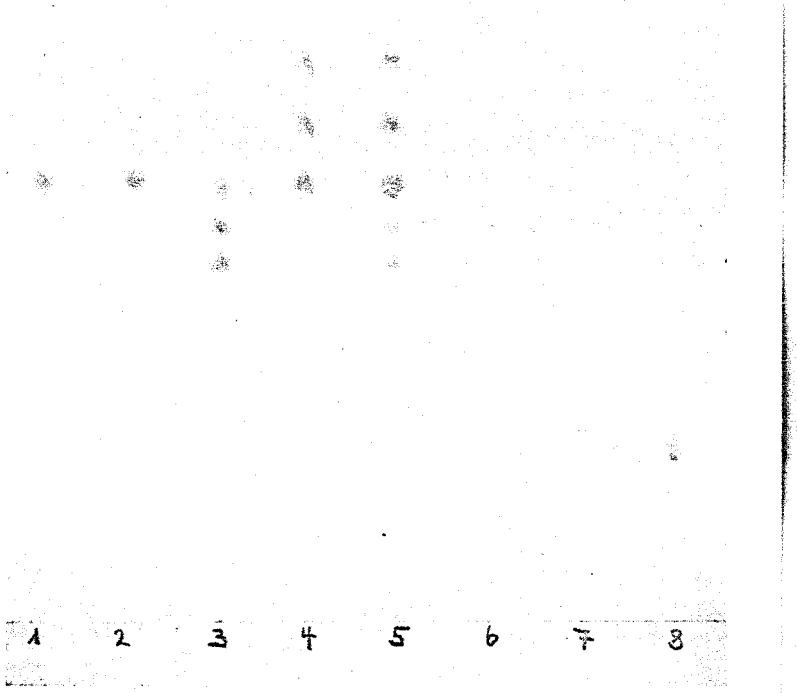

Figure 1

Thin Layer Chromatography of Sugars on a Layer of Cellulose Powder (Two-fold Chromatography in the System I).

(1) Mannose; (2) Arabinose; (3) Galactose, Glucose, Mannose; (4) Arabinose, Xylose, Ribose; (5) Mixture of 3, 4 and 6; (6) Rhamnose; (7) Glucoronic acid; (8) Galacturonic acid. For the pensoses 1.25 µg was applied each time. 2.5 µg of the remaining sugars was applied. The coloration was carried out with anisidine phthalate.

The cellulose powder can first be treated with a 0.5% solution of Versene /376 (ethylene diamine tetra-acetic acid) (removal of cations).

With this method, very small amounts of substance can be identified. The identification limit when coloration is carried out with anisidine phthalate is at 0.5 µg for hexoses and methylpentoses, and it is 0.1-0.2

µg for pentoses and uronic acids. Concentrations of approximately 2 µg are optimal for hexoses and approximately 1 µg for pentoses.

Quantitative evaluation experiments of the chromatograms are in process.

Institute for Chemistry and Physics*,
Federal Institute for Meat Research,
Kulmbach (Germany)

A. Schweiger

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Received June 21, 1962

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